

CLAIMS:

1. A method of assessing an amount of a known target nucleic acid sequence in a sample comprising:

- a) co-amplifying said target nucleic acid sequence and a known amount of a known control nucleic acid sequence to produce respective target and control amplicons, wherein said control nucleic acid sequence is different than said target nucleic acid sequence; and
- b) determining relative amounts of said respective amplicons by determining relative quantities of a primer extension reaction using each of said respective amplicons as a template,

wherein said primer extension reaction is performed using a sequential dispensation order of individual nucleotides, such that primer extension reactions of said respective target and control amplicons are performed sequentially of each other, and wherein determining relative quantities of a primer extension reaction comprises comparing a quantity of nucleotides incorporated during said primer extension reaction for said target amplicon with a quantity of nucleotides incorporated during said primer extension reaction for said control amplicon, wherein relative amounts of said respective amplicons are proportional to relative quantities of nucleotides incorporated during said primer extension reactions and said amount of said target nucleic acid sequence in said sample is proportional thereto.

2. The method of claim 1, wherein said control nucleic acid is an endogenous or exogenous nucleic acid.

3. The method of claim 1, wherein said primer extension reaction is performed using identical primers for said respective target and control amplicons.

4. The method of claim 1, wherein said primer extension reaction is performed using a different template-specific primer for said respective target and control amplicons.
5. The method of any of claims 1 to 4, wherein said primer extension reaction is detected by detecting pyrophosphate (PPi) release.
6. The method of claim 5, wherein said pyrophosphate is detected luminometrically.
7. The method of claim 6, wherein said pyrophosphate is detected enzymatically using the enzyme luciferase as a PPi-detection enzyme.
8. The method of claim 7, wherein in the primer extension reaction, an α-thio analogue of an adenine nucleotide is used.
9. The method of any of claims 1 to 8, wherein said target nucleic acid and control nucleic acid are co-amplified using amplification primers which are immobilized or carry means for immobilization.
10. The method of any of claims 1 to 9, wherein more than one different control nucleic acid is used.
11. The method of claim 10, wherein different amounts of each different control nucleic acid are used.
12. The method of any of claims 10 to 11, wherein said primer extension reaction is performed on said respective target and control amplicons using identical primers for said respective target and control amplicons.

13. The method of any of claims 10 to 11, wherein said primer extension reaction is performed on said respective target and control amplicons using a different template-specific extension primer on each said amplicon.

14. The method of any of claims 1 to 13, wherein multiple primer extension reactions are performed on each of said respective target or control amplicons.

15. The method of claim 14, wherein each said primer extension reaction yields an extension product of different length or sequence.

16. The method of any of claims 1 to 15, wherein said target nucleic acid is selected from the group consisting of a gene or a fragment thereof, a T-DNA region or a fragment thereof, a vector or a fragment thereof, a plasmid or fragment thereof, a nucleic acid polymorphism and a non-coding region of a nucleic acid sequence.

17. The method of 16, wherein said gene or fragment thereof is a gene selected from the group consisting of a disease resistance gene, an antibiotic resistance gene and a gene conferring an investigated trait.

18. A method of assessing copy number of a target nucleic acid sequence in a genome of an organism comprising:

- a) co-amplifying said target nucleic acid sequence and a known amount of a known control nucleic acid sequence to produce respective target and control amplicons, wherein said control nucleic acid sequence is different than said target nucleic acid sequence; and
- b) determining relative amounts of said respective amplicons by determining relative quantities of a primer extension reaction using each of said respective amplicons as a template,

wherein said primer extension reaction is performed using a sequential dispensation order of individual nucleotides, such that primer extension reactions of said respective target and control amplicons are performed sequentially of each other, and wherein determining relative quantities of a primer extension reaction comprises comparing a quantity of nucleotides incorporated during said primer extension reaction for said target amplicon with a quantity of nucleotides incorporated during said primer extension reaction for said control amplicon, wherein relative amounts of said respective amplicons are proportional to relative quantities of nucleotides incorporated during said primer extension reactions and copy number of said target nucleic acid sequence in said genome is proportional thereto.

19. The method of claim 18, wherein said control nucleic acid is an endogenous or exogenous nucleic acid.

20. The method of any of claims 18 to 19, wherein said target nucleic acid is selected from the group consisting of a gene or a fragment thereof, a T-DNA region or a fragment thereof, a vector or a fragment thereof, a plasmid or fragment thereof, a nucleic acid polymorphism and a non-coding region of a nucleic acid sequence.

21. The method of 20, wherein said gene or fragment thereof is a gene selected from the group consisting of a disease resistance gene, an antibiotic resistance gene, and a gene conferring an investigated trait.

22. A method of assessing copy number of a plasmid in a cell comprising:

- a) co-amplifying a target nucleic acid sequence from said plasmid and a known amount of a known control nucleic acid sequence to produce respective target and control amplicons, wherein said control nucleic acid sequence is different than said target nucleic acid sequence; and

- b) determining relative amounts of said respective amplicons by determining relative quantities of a primer extension reaction using each of said respective amplicons as a template,

wherein said primer extension reaction is performed using a sequential dispensation order of individual nucleotides, such that primer extension reactions of said respective target and control amplicons are performed sequentially of each other, and wherein determining relative quantities of a primer extension reaction comprises comparing a quantity of nucleotides incorporated during said primer extension reaction for said target amplicon with a quantity of nucleotides incorporated during said primer extension reaction for said control amplicon, wherein relative amounts of said respective amplicons are proportional to relative quantities of nucleotides incorporated during said primer extension reactions and said copy number of said plasmid in said cell is proportional thereto.

23. The method of claim 21, wherein said control nucleic acid is an endogenous or exogenous nucleic acid.

24. A method of identifying an organism having a single copy of a target nucleic acid sequence comprising:

- a) co-amplifying said target nucleic acid sequence and a known amount of a known control nucleic acid sequence to produce respective target and control amplicons, wherein said control nucleic acid sequence is different than said target nucleic acid sequence;
- b) determining relative amounts of said respective amplicons by determining relative quantities of a primer extension reaction using each of said respective amplicons as a template,

wherein said primer extension reaction is performed using a sequential dispensation order of individual nucleotides, such that primer extension reactions of said respective target and control amplicons are performed sequentially of each other, and wherein determining relative quantities of a primer extension reaction comprises comparing a quantity of nucleotides

incorporated during said primer extension reaction for said target amplicon with a quantity of nucleotides incorporated during said primer extension reaction for said control amplicon, wherein relative amounts of said respective amplicons are proportional to relative quantities of nucleotides incorporated during said primer extension reactions and said copy number of said target nucleic acid sequence is proportional thereto, and

- c) determining if said copy number of said target nucleic acid sequence is one, wherein a copy number of said target nucleic acid sequence equal to one is indicative of an organism having a single copy of said target nucleic acid sequence.

25. The method of claim 24, wherein said control nucleic acid is an endogenous or exogenous nucleic acid.

26. The method of any of claims 24 to 25, wherein said target nucleic acid is selected from the group consisting of a gene or a fragment thereof, a T-DNA region or a fragment thereof, a vector or a fragment thereof, a plasmid or fragment thereof, a nucleic acid polymorphism and a non-coding region of a nucleic acid sequence.

27. The method of 26, wherein said gene or fragment thereof is a gene selected from the group consisting of a disease resistance gene, an antibiotic resistance gene, and a gene conferring an investigated trait.

28. The method of any of claims 24 to 27, wherein said organism is a genetically modified organism.

29. The method of claim 28, wherein said organism is identified from a plurality of organisms.

30. The method of any of claims 28 to 29, wherein said organism is an organism selected from the group consisting of mammals, plants, birds, bacteria, fungi, and viruses.

31. The method of any of claims 1 to 30, wherein said control nucleic acid is acetohydroxyacid synthase (AHAS).

32. A kit for use in a method as defined in any one of claims 1 to 31, said kit comprising:

- a) a known control nucleic acid sequence of known concentration; and
- b) means for detecting a primer extension reaction.

33. The kit of claim 32, further comprising one or more of the following components:

- c) an extension primer for said control nucleic acid sequence and/or said target nucleic acid sequence
- d) a primer for amplification of said target nucleic acid;
- e) a primer for amplification of said control nucleic acid; and
- f) a polymerase enzyme for amplification of said target nucleic acid and said control nucleic acid, and/or for primer extension.

34. The kit of any of claims 32 to 33, wherein said target nucleic acid is selected from the group consisting of neomycin phosphotransferase (NPTII) phosphinothricin acetyl transferase (PAT), hygromycin phosphotransferase (HPT), acetolactate synthase (ALS, also referred to as acetohydroxyacid synthase (AHAS)), phosphomannose isomerase (PMI), 5-enolpyruvylshikimate-3-phosphate synthase (EPSP synthase), oxalate oxidase, adenosine deaminase, dihydrofolate reductase, thymidine kinase, xanthine-guanine phosphoribosyltransferase, and blasticidin S deaminase.

35. The method of any of claims 1 to 15, wherein said target nucleic acid is a chromosome or a fragment thereof and assessing an amount of said target nucleic acid is used to determine chromosome copy number.

36. The method of any of claims 1 to 31 and 35, wherein said target nucleic acid is selected from the group consisting of neomycin phosphotransferase (NPTII) phosphinothricin acetyl transferase (PAT), hygromycin phosphotransferase (HPT), acetolactate synthase (ALS, also referred to as acetohydroxyacid synthase (AHAS)), phosphomannose isomerase (PMI), 5-enolpyruvylshikamate-3-phosphate synthase (EPSP synthase), oxalate oxidase, adenosine deaminase, dihydrofolate reductase, thymidine kinase, xanthine-guanine phosphoribosyltransferase, and blasticidin S deaminase.

37. The kit of any of claims 32 to 34, wherein said control nucleic acid sequence is selected from the group consisting of acetolactate synthase, actin, profilin, ferredoxin-dependent glutamate synthase, ribose-phosphate pyrophosphokinase, beta-ketoacyl-CoA synthase, delta-5 fatty acid desaturase, sequence-tag sites (STS) of known copy number and non-translated sequences of known copy number.

38. The method of any of claims 1 to 31 and 36, wherein said control nucleic acid sequence is selected from the group consisting of acetolactate synthase, actin, profilin, ferredoxin-dependent glutamate synthase, ribose-phosphate pyrophosphokinase, beta-ketoacyl-CoA synthase, delta-5 fatty acid desaturase, sequence-tag sites (STS) of known copy number and non-translated sequences of known copy number.

39. The method of claim 1, wherein said primer extension reaction is performed using a primer specific for NPTII and another primer specific for AHAS.